THE EFFECT OF ETHER AND CHLOROFORM ON CERTAIN FISHES.[1]

HAROLD D. CLAYBERG.

I. INTRODUCTION.

Various drugs which cannot be standardized chemically must be standardized biologically before being used for medical purposes. The time required to kill an animal (for example, a goldfish) has been used for this purpose (see particularly Pittenger and Vanderkleed, 1915). The discussion about digitalis has been particularly keen. The effect of a large series of coal tar derivatives upon various wild fish has been studied by Shelford ('17) in connection with stream pollution work. He determined their relative toxicity by killing specimens of the orange-spotted sunfish. The work described below was undertaken with the two common drugs, ether and chloroform. Their effect on the orange-spotted sunfish and on certain other wild species was studied in the hope that it might serve to relate the coal tar results with those of the investigators interested in the standardization of drugs with domestic goldfish.

It is the purpose of this paper to point out that there seem to be three possible ways of standardizing a drug with fish.

1. Type of behavior in a gradient between pure water and water plus the drug being tested might be used. To furnish

definite standards a distinct form of behavior should appear or disappear at a definite concentration.

2. Time till definite symptoms of sickness appear might be used. Such a condition for serial concentrations is shown to the left in chart II.

3. Killing time is the only standard thus far applied.

In connection with killing it is our purpose to point out that the time-concentration curve is probably an hyperbola which, if fully demonstrated for a few substances, will render the investigation of drugs in general much easier.

II.   REACTIONS.

1. *Materials and Methods.*—The reaction experiments were performed in the Shelford gradient tank.   This is a tank 122 cm. long by 15 cm. wide by 13 cm. deep.   Water is allowed to flow in at each end at a definite rate.   It enters the tank through two tees, the cross bars of which are perforated with small holes so as to distribute the inflow throughout the width of the tank. It flows out at the middle at both top and bottom so the two kinds of water meet at the center.   In these experiments normal water was run in at the left end at a rate of about 450 c.c. per min. and normal water plus ether or chloroform was run in at the right end at an equal rate.   The drug solution was made up in an 18-liter bottle, placed above the apparatus, and siphoned out.   Siphon and bottle were closed on account of the volatility of the poisons used.   To be certain that the concentration under observation remained constant, the flows were tested occasionally throughout the experiment; hence the concentrations quoted are presumably accurate.   The outflow at the center did not prevent the mixing of the two kinds of water and thus the middle section equal to one half or third of the tank, was a gradient between the normal water and water plus ether or chloroform. Accordingly as a fish moved from left to right in the tank it encountered a gradually increasing concentration of the poison. We found no evidence that fish react to the slight current through the tank.   Since each half of the tank held about 9 liters, it required 21 minutes to fill it or to replace all the water in one of the halves.

Two 8 candlepower electric lights were fixed above the center of the two halves, *i. e.*, above a point midway between the screen partition in front of the introducer tees and the center drain. The lights were 15–20 cm. above the surface of the water which was 13 cm. deep. The tank and lights were enclosed under a black hood. The experiments were observed through openings in the hood above the lights or through the glass side late at night. Water differing as little as possible from that in which the fish usually live was used for control readings. Controls were observed and the conditions in the two ends of these were the same either because the water introduced at the two ends was alike or because no water was run into either end (standing water). Behavior of both bullhead and sucker in tank controls was of two types identified as *quiescence* and *circuiting*. The latter refers to swimming around the tank and involves both back and forth movement and crosswise movement. The crosswise movement, while ignored in the charts, should be understood to occur, especially in the case of controls. In controls quiescence predominated after a short period of circuiting (see Chart I., Bullhead Control).

In experiments of this kind the reactions of the fish are commonly graphed, that is the distance between two lines on a sheet of paper (see chart I.) is used to represent the length of the tank. Cross movements of the fish are ignored in the graphing. Vertical distance is used to represent time according to scale. Thus in chart I. control 3 the bullhead went the entire length of the tank twice during the first one minute and 20 seconds.

For the reaction experiments only suckers (*Catostomus commersonii*) and bullheads (*Ameiurus nebulosus*) were available, but their behavior is not materially different from that of the standard sunfish (*Lepomis humilis*). More fish were not obtainable on account of the swollen condition of the streams, hence the small number of experiments done.

2. *Reaction and Standardization.*—Standardization through reaction in gradients would be dependent upon (*a*) Definiteness of some change in reaction occurring (in case of the fish); (*b*) accuracy of the change with reference to concentration; (*c*) speed of standardization; (*d*) reasonably low cost of the process.

| BULLHEAD | SUCKER | BULLHEAD | BULLHEAD | BULLHEAD |
| Control 3 | Expt. A. 6. | Expt. B. 1. | Expt. B. 3. | Expt. B. 10. |
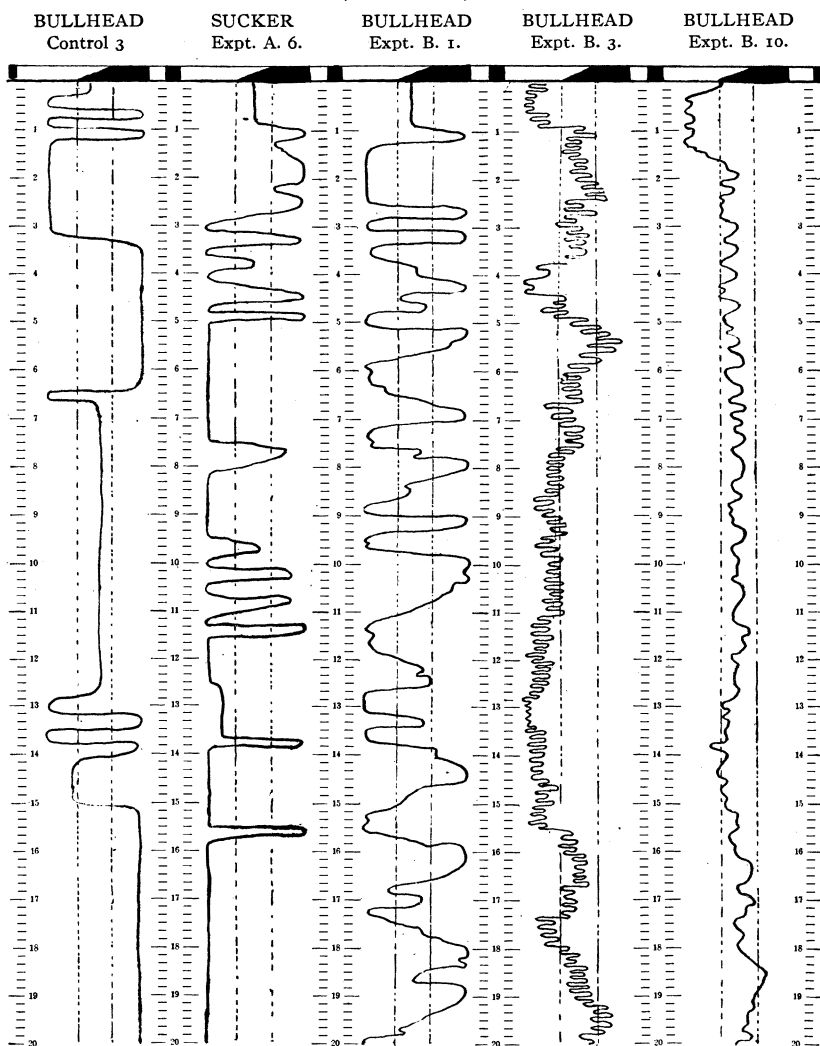
CHART I. Reaction graphs. The drug was introduced at the right; its concentration is indicated by the larger black area in the heading. These graphs represent parts of experiments only and are meant to show, primarily, the type of action of the fish to certain poison concentrations in the gradient tank. To the extreme left is shown a control experiment on a bullhead and it should be compared with the three to the right (of series B) in order to bring out the distinctive types of behavior involved. Not considering the sucker experiment and taking only those shown on the bullhead it is plain that an intergradation of action can be traced from the one on the extreme left to the one of the extreme right.

The reaction of the sucker to chloroform is shown in the second graph to the left and should be compared with the reaction of the bullhead to ether as shown in Expt. B. 1 for the concentrations are approximately isotoxic; the bullhead showing notches which are never present in the charts of suckers.

The following studies were carried out with the idea that a reaction might prove more definite, and perhaps less variable than time of death.

3. *Chloroform.*—Bullheads and suckers were used in a series of experiments which began with low concentrations which were increased in the successive trials. Both species showed considerable individual variation and in general failed to react to most concentrations. Both species showed some positive reactions in the case of .14 c.c. per liter (213.8 mg.) and a few negative reactions occurred in somewhat higher concentrations. There appeared to be no definite relation between reaction and fish size. The general failure to give definite positive or negative reactions may be due to a paralysis of the sensory endings with which fishes are known to recognize substances in solution in water.

4. *Ether.*—Bullheads alone were used, but a complete series of ten experiments were run in which concentration ranged from .267–4.573 c.c. per liter (192.24–3292.56 mg.). These solutions were all distinctly non-fatal because no symptoms of even partial loss of equilibrium were observed. Although some experiments ran for three hours, the fish exhibited no true positive or negative reaction when any experiment as a whole was considered. If, however, a small piece of one were carefully chosen an impression of reaction in either direction could be obtained from any experiment. The piece chosen from experiment B. 3 on chart I. was chosen for another purpose but it appears to show negative reaction. This is not the case as the whole experiment rather points to indefinite reaction.

While tank end preference was nil, the general behavior of the fish underwent definite change twice during the gradual increase in strengths of the poison used. Chart I. contains critical experiments of which repeated mention will be made through the rest of the reaction discussion. Experiment B. 1 was the first of the series and shows that circuiting predominated; but occasional little notches occur in the curve that are absent in the sucker's curve (exp. A. 6) and which are very suggestive of the behavior of the bullhead to higher concentrations. Thus experiment B. 1, having a concentration of .267 c.c. per liter (192.2 mg.), fixes a

definite sort of behavior for comparison with later experiments.

After 100 minutes in this .267, or after 13 minutes in .403 (290.1 mg.) a very distinct third type of reaction appeared. This might very appropriately be named the *nosing reaction*. It was characterized by incessant restlessness and continual change of direction; the fish nosed back and forth in a peculiar uneasy manner, turning around 180° every 2–8 seconds, holding its head always close against the glass side of the tank, and keeping this up for hours. Experiment B. 3 shows how remarkably this reaction differed from the others. In concentration 4.573 (3,292.5 mg.) this nosing reaction was entirely eliminated, and a still different type of reaction appeared (see exp. B. 10).

This reaction might well be designated as *intermediate*, since it seemed to be a compromise between the circuiting of exp. B. 1 and the nosing reaction of higher concentrations, in that it exhibited the slow turning of the former combined with the narrow range of the latter. The range of the nosing reaction in relation to concentration is shown in Table I. It seems that such an apparently precise reaction ought to be found with fish or other aquatic animals in the case of drugs needing standardization. Comparison of Table I. with parts of three experiments shown to the right in chart I. will make clear the significance of the results attained.

TABLE I.

SHOWING RELATION BETWEEN ETHER CONCENTRATION AND TIME TILL INCEPTION OF THE NOSING REACTION IS OBSERVED.

Series B. Exp. Number.	Poison Entry Rate in Cu Mm./Sec.	Poison Concentration at Right End of Tank.		Time till Nosing Reaction Started, Minutes.	Duration of Expt., Minutes.
		C.c. per Liter.	Mg. per Liter.		
1	2	.2667	192.0	100	128
3	3	.6667	480.0	30	182
5	10	1.3333	960.0	10	86
9	30	3.7855	2725.6	0	97
10	35	4.5733	3292.6	*	72

* No nosing reaction whatever occurred throughout this experiment.

III. RESISTANCE.

1. *Materials and Methods.*—Killing of fish was carried on in closed bottles in which poison solutions of known concentrations were made up. As a rule 4-liter bottles were used, although

some of 2- and 5-liter were also utilized. The fish was placed in the solution after being measured and weighed; and the time till its behavior became definitely abnormal and the time till death were carefully noted.

2. *Results with the Minnow* (*Pimephales*).—Preliminary work was undertaken looking towards a comparison of the closed bottle method of killing (used in all drug standardization work) with that of running poison of known concentration at definite rate through a bottle. The results indicated that the two methods had like effect for the same poison concentrations. Further, the results showed that the hour killing concentration for the minnow was between 2.2 and 2.3 c.c. per liter for ether (1,584–1,586 mg.). Weight used varied from 1.5–4 grams.

3. *Ether: The Sunfish.*—The standard used by Shelford for the measurement of toxicity of poisonous polluting substances is the concentration of any poison sufficient to kill a 4–5 gram *Lepomis humilis* in approximately 60 minutes. The hour killing concentration for ether was found to be between 3.685 and 3.690 c.c. per liter (2,653.2–2,656.8 mg.). Apparatus at hand permitted no closer determination. The number of experiments run was sufficient to plot the death curve shown in chart II. The base line or X-axis was used for minutes until the fish became abnormal or dead. The Y-axis was used for the concentrations employed. Points for all experiments were plotted and these were connected to form a curve. This curve showed a certain width due to variation in time of death throughout, which has been brought out by stippling.

The data were now separated into three classes to enquire into the effect of fish weight on death time. Since not enough fish of strictly standard size could be obtained, fish of size between 2 and 25 grams were used. Those obviously outside the weight from external appearance were used to determine the critical concentration roughly and those nearest the proper standard were used last. These were now placed in three classes: class 1, 2–9 grams; class 2, 10–19 grams; class 3, 20–25 grams. These were plotted in three curves on chart II. Those of classes 1 and 2 were complete enough to show the following facts.

At about 4.8 c.c. per liter (3,456 mg.) the fish of the two classes

CHART II.

Death Curve of Lepomis Humilis in Ether.

$((C_2H_5)_2O.)$

Tenth c.c.
per liter

70

68

66

64

62

60

58

56

54

52

50

48

46

44

42

40

38

36

34

32

Class 1.

class 2

Class 2

Class 3

class 1

class 1

Class 2

Class 1

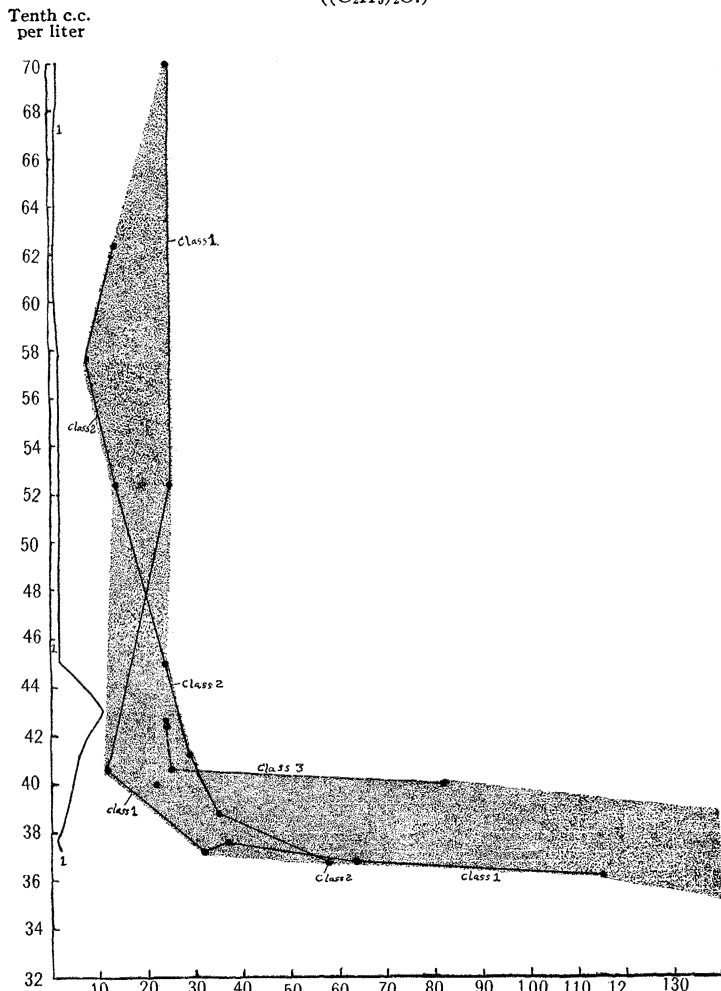10 20 30 40 50 60 70 80 90 100 110 12 130

CHART II. (Ether death curve.) The curve to the left is the "abnormal curve" and marks the inception of symptoms such as loss of balance. The obvious fall in toxicity below 420 cu. mm. per liter is shown by the stippled death curve turning off to the right. Class 1 was of fish 2–9 grams in weight; class 2 was of fish 10–19 grams in weight; class 3 of fish 20–25 grams in weight. The significance and curve directions are discussed in the text. It is very likely there would have been much intercrossing if many experiments had been run and ten or more classes had been separated. It may be that difference of physiological condition of different batches of fish (as chance allots in the purchase of fish for standardizing) may give results with the same concentration of the same drug as far apart as the different weights of fish here have done. In the case of the fish used here all were caught in the same place, and kept in constant environment in the same tank until used.

The upper and lower boundaries of the curve at the lower right hand edge of the cart were determined by experiments not shown in the graph. I am indebted to Miss Dorothy Wing, of the University of Chicago, for aid in the preparation of this chart.

died in the same time.   Above that concentration the toxicity
of the poison increased for the 10–20 gram fish and decreased
for the 2–9 gram fish up to about 5.3 c.c. per liter (3,816 mg.),
beyond which it remained constant.   Below about 4.8 c.c. per
liter, on the contrary, the toxicity of ether increased in the case
of class 1, and decreased in class 2 to a maximal difference at
about 4.1 c.c. per liter (2,952 mg.) below which the two converged
until they met at 3.7 (2,664).   Their crossing point is at the
hour death concentration.   If, at certain points, the death
curves of different weights of fish are found to cross, in standard-
izing aconite, for example, it would be well to take these points
as the standard and disregard the hour standard or any other
previously set.

The third class furnished complete data only between 4 and
4.35 c.c. per liter (2,880 to 3,132 mg.).   At the upper concen-
trations the toxicity is intermediate between classes 1 and 2.
But below 4.1 c.c. per liter (2,952 mg.) the toxicity falls away
far more rapidly than for the others.   It is very likely that
concentrations below 3.9 (2,808 mg.) will cease to have toxic
effect of any sort on class 3.   Since the critical concentration
is well below this point the significance of weight of the fish used
is seen to be of the utmost importance.   It is regrettable that
no large fish were available to test out the end of the class 3
curve.   Very small fish below 2 grams are, as might be expected,
killed in much less time than classes 1 or 2.   Thus the resistance
of the fish is seen to be consistently different for different weights
and also of considerable complexity.

The crossing of the curves of classes 1 and 2 renders both
classes of data available in the determination of the hour death
point.   The above results point out that, in standardization of
drugs by fish, the different sizes may well prove to differ con-
sistently and considerably.   For this reason it would seem that
the weight problem needs careful analysis and extensive experi-
mentation to determine the proper weight limits to be allowed
for results of the accuracy demanded.

Examination of the fish led to the graphing on the same sheet
(see chart II.) of the time it took the fish in any concentration to
become definitely abnormal (loss of balance, etc.).   Above 4.5

c.c. per liter (3,240 mg.) the time was 1–2 minutes but below this concentration there occurred either a sudden decrease in initial toxicity or an equally strong increase in the resistance of the fish. This culminated at 4.3 c.c. (3,096 mg.) per liter in an "abnormal" time of 11 minutes, and from there, with further *decrease* in strength of solution the initial toxicity *increased* to the norm (1–2 min.) at a concentration of 3.7 c.c. per liter (2,664 mg.).

If a relation such as this be found for fish in standardizing some drug, it will be both quicker and cheaper to use it instead of death. Decreased initial toxicity with decrease in strength and close approach to hour killing concentration would be in no wise remarkable if it did not increase again to the norm as the concentration fell to the hour killing concentration as determined. But enough fish were used to render this inexplicable phenomenon reasonably certain.

4. *Chloroform: the Sunfish.*—The series run here were also plotted as explained for ether. See chart III. The hour death concentration lies between about .07 and .1 c.c. per liter (106.89 and 152.7 mg.) and much variation is exhibited between these points. The rest of the curve shows no such variation but it may be that this was due to the small number of experiments used to determine them. It is equally likely that only certain limited regions exhibit variability. Should this be the case in a drug needing standardization the variable areas should be located and no standard set anywhere within one of them. Continuous variation throughout a curve would, if wide enough, forbid the use, for accurate standardization work, of the experimental animal used in making the curve.

IV. Comparison of Ether and Chloroform Killing Curves.

It has been stated that these curves are hyperbolæ or very close to them. It should be further noted that the maximum death variability seems to coincide with that part of the curve showing greatest curvature. The laws governing hyperbolæ will probably be found to be of great use in fish standardization since they seem to die according to one. Twenty-four experi-

CHART III.

Death Curve of Lepomis Humilis in Chloroform.
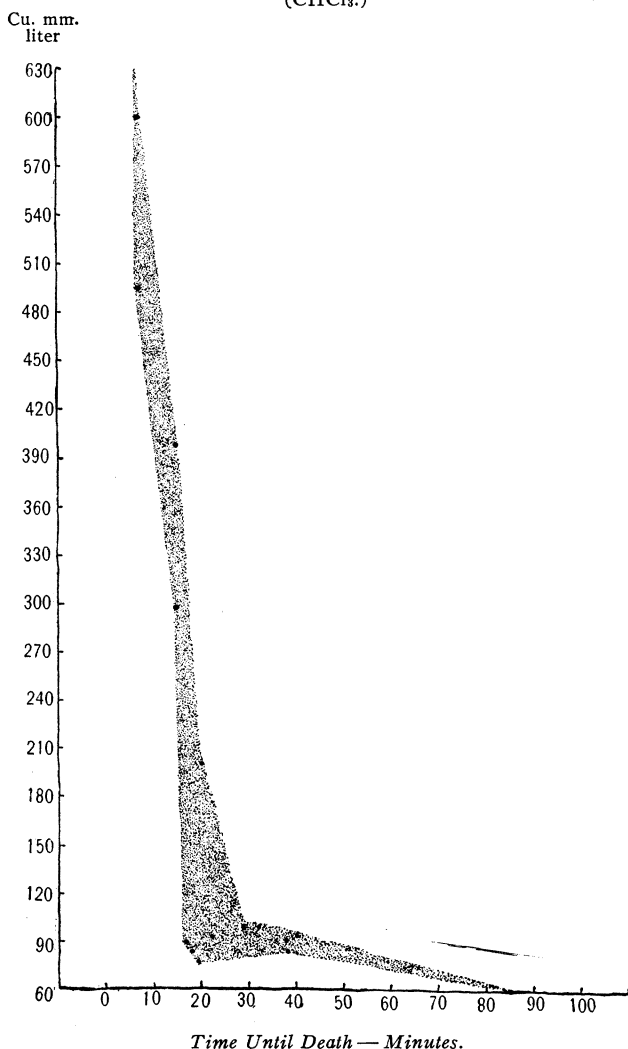
(CHCl₃.)

Time Until Death — Minutes.

CHART III. (Chloroform death curve.) Each point represents a separate experimental result. The poison is obviously very toxic as a concentration of less than .09 c.c. per liter kills in an hour. There is a sudden change in the curve between the concentrations of 100 and 200 cu. mm. per liter as if a new factor suddenly entered into the determination of the curve below the limits indicated. Only four of the fifteen fish used were above 6.2 grams and these were all used to determine the curve above 190 c. mm./liter. Hence no classes by weight could be separated.

ments were used to determine the ether curve and 15 for that of chloroform.   This does not seem to be sufficient data to establish a law although it appears obvious that there may be one here. The upper limb of the chloroform curve is nearly a straight line and in it $c \times t = k$, about 4,200, c being concentration in cu. mm. per liter and t being time until death.   It is probable that time, weight of fish, and concentration of poison used, will prove the determining factors for relative toxicity.

The similarity of the chloroform and ether curves suggest that after the location of a toxic zero, Krogh's ('14) Law may be found to govern under certain limitations.   Should a law be here discovered and prove to hold closely enough, it is easily seen that it will prove of very great value in the standardization of poisons by means of fish.

V.   Poisoning Symptoms.

1. *Ether.*—No particular irritability or spasmodic movements were noted.   The fish at first acted normal, then swam vertically with head up, lastly quieted down and lay on its back or side, very usually at the surface of the water.

2. *Chloroform.*—For anæsthesia with either chloroform or ether Cushny[1] notes three stages: (1) Imperfect consciousness, (2) excitement, (3) anæsthesia.   In fish, although the first stage is almost impossible to distinguish from the normal, the others may be definitely noted.   The second stage is characterized through all concentrations used by a variable period of violent muscular contraction, which throws the fish wildly in all directions so that it bumps its nose strongly on the glass each time.   Each bump is followed by a motionless pause of one half to one second. The third stage, coma, is reached in a time increasing as the concentration decreases.   The fish lies on its side, or rarely on its back, at the bottom of the jar and the opercular movement is regular at first, then intermittent, and finally ceases.   While the fish respires regularly it may recover if held under a stream of running water.

3. *Comparison of Ether and Chloroform.*—(*a*) In ether the stage of excitement is short or omitted while in chloroform it is

[1] Pharmacology, p. 151.

obvious and protracted. (b) In the sunfish ether produces good anæsthesia allowing easy recovery, while chloroform poisons rather than anæsthetizes. (c) Honigman[1] states that with man chloroform is approximately three times as strong as ether (by weight). With the sunfish it is about 44 by volume or 20.4 by weight. (d) From previous statements it might be well to add that, if death remain the biologic change used for standardization, in certain cases it may be that other killing times may prove more accurate than the 60-minute standard.

4. *Comparison of Ether and Chloroform with Coal Tar Derivatives.*—Shelford ('17) has investigated the effect of various coal tar derivatives on standard fish. In comparing these poisons with the drugs previously discussed in this paper the following method was used. The poisons were put up in concentrations twice as strong as would be necessary to kill in an hour with the object of killing the fish in approximately 15 minutes. A specimen of golden shiner (*Abramis chrysoleuca*) was put into a 2-liter bottle of each poison solution and when it was dead a second was inserted. The actions of the fish were noted carefully. In all cases the opercular movement was greatly increased in amount and rate at the beginning of stage 2 (excitement) and later weakened and decreased. The action of these coal-tar derivatives as irritants and poisons was like that of chloroform but distinctly different from that of ether. A large amount of blood appeared to collect at the ventral side of the body toward the end of stage 2. This was notably so in quinoline, less so for aniline and toluene, and observable in the others. No such phenomenon was observed in the parallel chloroform and ether bottles.

TABLE II.

Poison Used.	Concentration in Cu Mm./Liter.	Time Different Fish Were Killed In.	Relative Irritability.	Opercular Rate Range Observed During Experiment
Toluene........	150	15, 23	Highest at first.	60– 88
Quinoline......	100	12, 20	Longest.	27–144
Aniline........	2,000	8, 9, 10	Very strong.	30– 60
Orthocresol.....	100	47	Medium.	70–240
Chloroform.....	600	7, 18	Strong.	22–180
Ether..........	4,250	18, 20	Very little.	90–102

[1] Cushny, *op. cit.*, p. 163.

VI. Summary and Conclusions.

1. The behavior of bullhead and sucker in gradient tank to chloroform was usually indefinite and followed no rule.

2. Bullheads exhibit a peculiar nosing reaction in ether concentration (in gradient tank) between .3 and .4 c.c. per liter but not above or below these limits (except as noted). Such definite and remarkable changes in behavior may be determining factors in use of fish for standardization of poisons.

3. Concentrations of about .07 c.c. (106.89 mg.) per liter chloroform or of 3.69 c.c. (2,656.8 mg.) ether can kill standard sunfish in an hour.

4. In ether during third stage of anæsthesia the fish usually lies belly up at the surface while in chloroform it tends to lie on its side at the bottom of the bottle.

5. Toxicity ratio between chloroform and ether is 3 : 1 (by wt.) for man and 20 : 1 (by wt.) for the sunfish. Such constants may be of critical value in comparing the relative usefulness of different animals for standardization uses. It may not be the animal nearest man as regards toxicity ratio which is most valuable for standardization. For one where the toxicity ratio is far greater, and hence an animal far more susceptible, may be most accurate in perceiving and reacting to, or in dying from, the poison demanding measurement. Nearness or likeness to man is of little account and precision of reaction or death the paramount issue.

6. Ether anæsthetizes the sunfish while chloroform acts more as a poison, being, in a certain way, intermediate between ether and the violently irritating coal-tar derivatives studied above and in Shelford's work.

I take this opportunity to express my thanks to Professor Victor Shelford, of the University of Illinois, for the courteous help and kindly encouragement which made this paper possible.

VII. BIBLIOGRAPHY.

Chase, C. S.
'16 Physiological methods of drug standardization. J. Amer. Pharm. Assn. v. 4, p. 1289.

Eckler, C. R.
'12 Experiments with the cat method for testing digitalis and its allies. J. Amer. Pharm. Assn., v. 1, p. 715.

Eggleston, Chas.
'13 Biological standardization of the digitalis bodies by cat method of Hatcher. Am. J. Pharm., v. 85, p. 99.

Ford, J., and Wine, C. S.
'15   Comparison of the guinea pig and cat methods for the standardization of aconite.   Am. J. Pharm., v. 87, p. 489.

Gottlieb, R.
'14   Standardization of digitalis preparations on the frog.   Muenchener med. Wochenschrift, v. 61, p. 813.

Hale, Worth.
'12   Seasonal and sex variations of Rana pipiens to digitalis poisoning.   J. Pharmacology, v. 3, p. 458.

Haskell, Chas. C.
'12a   Physiological drug testing and the pharmacopœia.   J. Amer. Pharm. Assn., v. 1, p. 696.
'12b   Seasonal variations in the resistance of the guinea pig to poisoning.   Am. J. Pharmacy, v. 84, p. 241.
'13   Seasonal variations in the resistance of guinea pigs to poisoning.   Lilly Sci. Bull., v. 3, p. 80.

Haskell, C. C., and Eckler, Chas. R.
'13   Tentative standards for some biologically standardized drugs.   J. Am. Pharm. Assn., v. 2, p. 146.

Haskell, C. C., and Thomas, H. B.
'16   A comparison of the guinea pig and cat methods in the testing of aconite.   Am. J. Pharm., v. 88, p. 3.

Krogh, A.
'14   On influence of temperature on rate of embryonic growth.   Zeit. f. Allg. Phys., 16, 163–177.

Pittenger, Paul S., and Vanderkleed, Chas. E.
'15   Preliminary Notes on a New Pharmacodynamic Assay Method.   Jour. of Amer. Pharm. Ass., 4, 427–433.

Saylor.
'16   Drug standardization by means of lupine seedlings.   Am. J. Pharm., v. 88, p. 8.

Shelford, Victor E.
'16   Physiological Characters of Marine animals from different depths.   Puget Sd. Mar. Sta. Pub. v. 1, p. 157.

Shelford, V. E.
'17   An experimental study of the effects of gas waste upon fishes, with especial reference to stream pollution.   Bull. Ill. State Lab., Vol. xi, p. 381–412.

Shelford, V. E., and Allee, W. C.
'13   Reactions of fishes to gradients of dissolved atmospheric gases.   Journal Exper. Zool., v. 14, p. 207.
'14   Rapid modification of the behavior of fishes by contact with modified water.   Journal Animal Behavior, v. 4, pp. 1–30.

Shelford, V. E., and Powers, E. B.
'15   Experimental study of the movements of herring and other marine fishes.   BIOL. BULL., v. 28, p. 315.

Vanderkleed, Chas. E.
'12   Variation in the susceptibility of the guinea pig to the heart tonic group.   (a) J. Am. Pharm. Assn., v. 1, p. 701; (b) Am. Jour. Pharm., v. 84, p. 14.

Walters, R., and Haskell, C. C.
'12   Susceptibility of the guinea pig to poisoning by digitalis.   J. Amer. Pharm. Assn., v. 1, p. 709.

Weis, Edmund.
'12   Physiological standardization of digitalis preparations.   Pharm. Post., v. 45, pp. 429 and 441.